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EFFECT OF BICARBONATE AND OTHER BUFFERS ON CHOROID PLEXUS Na^+/K^+PUMP

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Summary

The Na^+/K^+ pump on the apical membrane of the choroid plexus epithelium was found to be sensitive to bicarbonate. Glycodiazine, a non-volatile, lipid soluble buffer with a pK of 5.7, mimicked the effect of bicarbonate, and was transported across the epithelium in the same direction as sodium. These results are explained in terms of a Na^+/H^+ exchange mechanism on the basal-lateral membrane.

It is a common observation that bicarbonate stimulates active salt and water transport across epithelia. In the frog choroid plexus HCO_3^- (25 mM) doubles the rate of active sodium secretion, and HCO_3^- appears to follow Na^+ into the cerebrospinal fluid (CSF) in order to maintain electroneutrality [1,2]. To clarify the role of HCO_3^- in CSF secretion I have studied the effect of this and other buffers on the Na^+/K^+ exchange pump located on the apical membrane of the choroidal epithelium [1,3,4].

As an index of Na^+/K^+ pump rate I measured the ouabain-sensitive $^{42}K^+$ flux across the apical membrane from the CSF into the epithelium. The method was similar to that described previously for iodide transport [5]. In brief, it was to measure the amount of the isotope in the epithelium after exposing the apical membrane for short periods of time (0.5—4 min) to Ringer's solutions containing $^{42}K^+$. The amount of $^{42}K^+$ trapped on the surface was estimated by including an extracellular marker ([^{3}H] mannitol) in the Ringer's solutions. The uptake of potassium was linear for at least two minutes; this suggests that uptake is a valid measure of unidirectional influx of potassium across the apical plasma membrane. Unidirectional fluxes of ions across the epithelium were measured as described elsewhere [1]. The Ringer's solutions contained 105.5 mM NaCl, 2 mM KCl, 1 mM $MgSO_4$, 1 mM $CaCl_2$ and were buffered to pH 7.3 with 2.5 mM sodium phosphate.

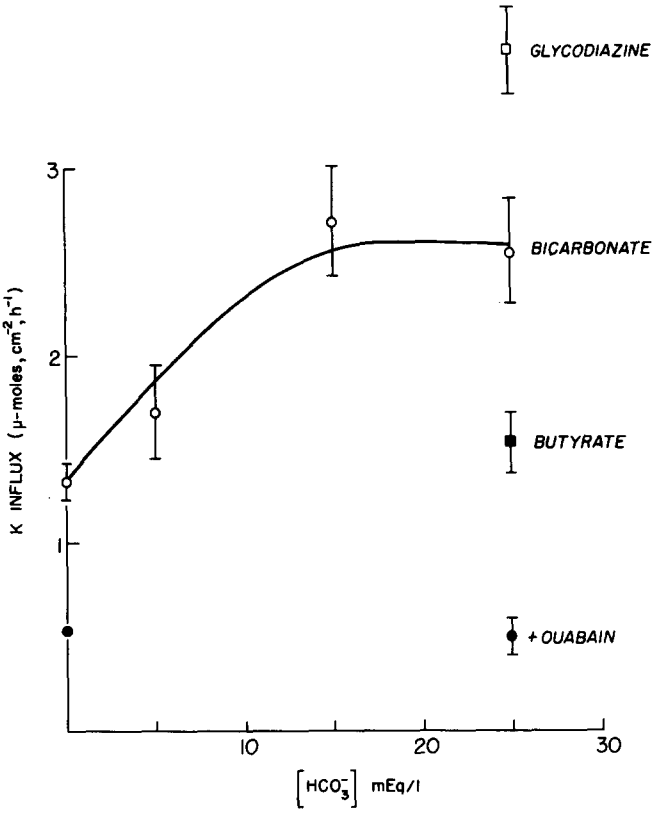

Fig. 1. The potassium unidirectional influx across the apical plasma membrane of the frog choroid plexus as a function of buffer composition. The influx was obtained from the $^{42}K^+$ influx over a 2-min incubation period as described in the text. The ouabain concentration was $7 \cdot 10^{-6}$ M, and all data points shown are means of three to six experiments with the standard errors. Note that in all experiments the pH of the Ringer's solution was 7.3.

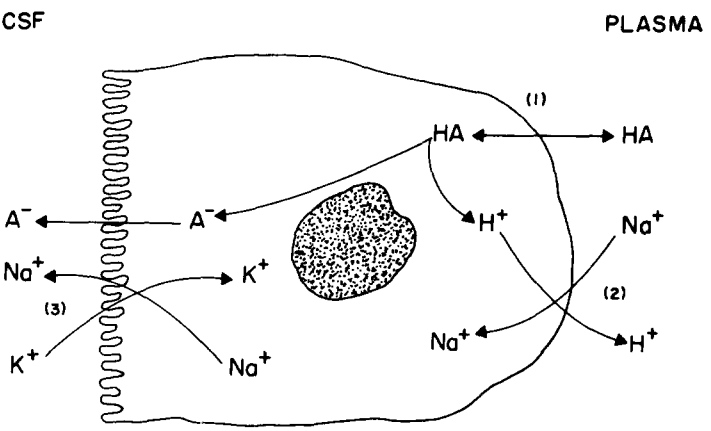

Fig. 2. A model for the effect of buffers on sodium transport across the choroid plexus: 1. Buffer enters the cell across the basal-lateral membrane by non-ionic diffusion; 2. Protons liberated intracellularly from dissociation of the buffer exchange with Na^+ in plasma; and 3. The increase in intracellular Na^+ stimulates the Na^+/K^+ pump at the apical surface of the cell. Buffer anion accompanies Na^+ into the CSF and accounts for the difference between the sodium and chloride composition of the freshly secreted fluid.

In some experiments NaCl in the Ringer was partially replaced with $NaHCO_3$, sodium glycodiazine (Redul®: *N*-(5-(2-methoxyethoxy)-2-pyrimidinyl) benzensulfonamide) or sodium butyrate. Bicarbonate-free solutions were equilibrated with 100% O_2, and the bicarbonate solutions were gassed with appropriate CO_2/O_2 mixtures to maintain the pH at 7.3.

In the presence of 25 mM HCO_3^- the unidirectional influx of potassium across the apical membrane of the epithelium was 2.7 ± 0.2(13) μmol cm^{-2} h^{-1}. Ouabain ($7 \cdot 10^{-6}$ M) in the CSF reduced the influx by 80%, and ouabain together with ethacryanic acid ($1 \cdot 10^{-3}$ M) eliminated all but 3% of the influx. The effect of HCO_3^- on the potassium influx is shown in Fig. 1. The flux doubled as the HCO_3^- concentration increased from 0 to 25 mM, and then remained constant up to 65 mM HCO_3^-. The effect of ouabain at 0 and 25 mM HCO_3^- indicates that bicarbonate directly stimulates the Na^+/K^+ exchange pump.

The relative effects of 25 mM HCO_3, butyrate, and glycodiazine on the Na^+/K^+ pump are also shown in Fig. 1. Glycodiazine (p*K* 5.7) was more effective in stimulating K^+ transport than either bicarbonate (p*K* 6.3) or butyrate (p*K* 4.8); in fact, it is doubtful whether or not butyrate stimulates the pump above the level obtained with the control phosphate buffer. In the renal proximal tubule, butyrate and glycodiazine are as effective as HCO_3^- in promoting fluid absorption [6], while in the pancreas, glycodiazine only partially maintains secretion [7].

In the presence of bicarbonate sodium is secreted across the plexus into the CSF (Fig. 2), but the rate of chloride transport is only about half the rate of sodium transport [1,2]. Although, there is circumstantial evidence that bicarbonate ions make up the difference between the rates of sodium and chloride transport, it is difficult to measure the bicarbonate flux directly owing to the volatile nature of the buffer. This problem does not arise with glycodiazine, which can effectively mimic bicarbonate in maintaining the Na^+/K^+ pump rate in this tissue. Using [^{3}H]glycodiazine (specific activity 88 mC/mM) I have measured the fluxes of the buffer across the plexus as described for other solutes. At a concentration of 25 mequiv./l the glycodiazine unidirectional flux from blood into the CSF was 0.8 ± 0.1(7) μmol cm^{-2} h^{-1}, while backflux was only 0.5 ± 0.05(6) μmol cm^{-2} h^{-1}, i.e. there was a net flux of 0.3 μmol cm^{-2} h^{-1} into the CSF. This net anion flux can account quantitatively for the deficit between the sodium and chloride transport rates into the CSF. Ouabain ($1 \cdot 10^{-5}$ M) in the CSF abolished the net transport of glycodiazine; the unidirectional flux from blood to CSF was reduced by 0.28 ± 0.10(7) μmol cm^{-2} h^{-1} while there was no significant change in the backflux into blood from the CSF. Ouabain at this concentration blocks active sodium secretion by the plexus. Glycodiazine transport across the renal proximal tubule is sodium sensitive and partially blocked by ouabain [8,9].

These experiments show that bicarbonate and glycodiazine increase the Na^+/K^+ pump rate across the apical membrane of the frog choroid plexus. The most probable explanation is that buffers such as bicarbonate stimulate the Na^+/K^+ pump rate indirectly by increasing sodium entry into the cell across the basal-lateral membrane. This is represented in Fig. 2

by three steps. First, lipid soluble buffers with pK values around 6 can enter the epithelium by non-ionic diffusion where they dissociate to liberate protons and buffer anions. (In the case of bicarbonate, diffusion of CO_2 and the catalysed hydration of CO_2 by carbonic anhydrase play an additional important role in the supply of intracellular protons and buffer anions.) Second, protons are transported back out of the epithelium by a H^+/Na^+ exchange mechanism on the basal-lateral membrane of the epithelium. Sodium entry down its electrochemical potential gradient, ~135 mV, is therefore increased in the presence of buffers owing to the rise in supply of intracellular protons and the exchange of Na^+ for H^+ across the basal-lateral membrane. In the proximal tubule and intestine there is ample evidence for Na^+/H^+ exchange at the brush border membrane of the epithelium [9,10]. Finally, as the intracellular sodium concentration increases, this stimulates the Na^+/K^+ pump on the apical membrane, and the buffer anion follows Na^+ across the apical membrane into the CSF. This scheme explains HCO_3^- transport and stimulation of Na^+ transport without the need to postulate plasma membrane HCO_3^--ATPase. In fact, our studies [11] of rat intestinal epithelium show that HCO_3^--ATPase is not associated with plasma membranes.

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